

Appl. No. 10/006,011
Amdt. dated November 4, 2003



PATENT

Attorney Docket No.: 8321-95US



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re:	Patent application of	:	
	Renato V. Iozzo	:	
		:	Group Art Unit:
Serial No.:	10/006,011	:	1642
		:	
Filed:	December 4, 2001	:	Examiner:
		:	Christopher H. Yaen
For:	ENDOREPELLIN: METHODS AND	:	
	COMPOSITIONS FOR INHIBITING	:	
	ANGIOGENESIS	:	

Declaration of Renato V. Iozzo, M.D. under 37 C.F.R. 1.132

Mail Stop RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I, Renato V. Iozzo, declare:

1. I am the inventor in the above-referenced patent application.
2. I hold an M.D. degree from the University of Florence School of Medicine, Florence, Italy, and have completed residencies in pathology at both the Institute of Pathology, University of Florence and the Department of Pathology, University of Washington. I was also a Senior Fellow in the University of Washington Department of Pathology. I am licensed to practice in the states of Washington and Pennsylvania. I am

CERTIFICATE OF MAILING UNDER 37 C.F.R. 1.8(a)	
I hereby certify that this paper, along with any paper referred to as being attached or enclosed, is being deposited with the United States Postal Service on the date indicated below, with sufficient postage, as first class mail, in an envelope addressed to: Mail Stop RCE, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450	
BY	<i>Shanna R. Smith</i>
DATE:	2/4/04

board certified in Pathology by both the Italian Board of Pathology, University of Parma, Italy and the American Board of Pathology.

3. I am a Professor of Pathology, Anatomy, and Cell Biology at Thomas Jefferson University, Philadelphia, Pennsylvania and a member of the Kimmel Cancer Center of Thomas Jefferson University. I am an Attending Pathologist and Director of Electron Microscopy at Thomas Jefferson University Hospital. I am the author or co-author of over 200 peer-reviewed articles and abstracts. I have over twenty years experience studying the structure and biology of the extracellular matrix and have training and expertise in various aspects of cellular and molecular biology.

4. I have read and understand the Office Action mailed November 4, 2003. I understand that the Examiner has rejected claims for lack of written description, enablement and obviousness.

5. I understand that this Declaration is to be submitted to the U.S. Patent and Trademark Office as part of the Response to the Office Action mailed November 4, 2003.

6. I declare that the human perlecan protein recited in the specification as filed is same the human perlecan whose amino acid sequence is provided in Murdoch et al. (J. Biol. Chem. 1992, 267:8544), an article co-authored by me and published by my laboratory. The Murdoch et al. paper is referenced in the specification as filed.

7. I declare that the perlecan amino acid sequence added to the amended Sequence Listing submitted herewith as SEQ ID NO:2, is the same perlecan amino acid sequence published in Murdoch et al.

8. The amino acid residue position designations of endorepellin and endorepellin fragments $\Delta 1$, $\Delta 2$, $\Delta 3$, $\Delta 4$, $\Delta 5$, $\Delta 6$, and $\Delta 7$ as described in the specification as filed, are based on their respective amino acid residue positions in perlecan.

9. I declare that the amino acid sequences of endorepellin and endorepellin fragments $\Delta 1$, $\Delta 2$, $\Delta 3$, $\Delta 4$, $\Delta 5$, $\Delta 6$, and $\Delta 7$ added to the amended Sequence Listing submitted herewith, have been extracted from the perlecan amino acid sequence, based upon the amino acid residue position information provided in the specification as filed.

10. I declare that new SEQ ID NOS:2, 3, 4, 5, 6, 7, 8, 9, and 10 added to the amended paragraph on page 19 of the Specification, correspond to the amino acid sequences for perlecan, endorepellin, and fragments $\Delta 1$, $\Delta 2$, $\Delta 3$, $\Delta 4$, $\Delta 5$, $\Delta 6$, and $\Delta 7$, respectively, of the amended Sequence Listing.

11. I present below studies performed by me or under my direct supervision showing that endorepellin fragment $\Delta 7$ (SEQ ID NO:10): 1) has anti-angiogenic activity; and 2) that endorepellin (SEQ ID NO:3) and endorepellin fragment $\Delta 7$ (SEQ ID NO:10) cause clustering and co-localization of the $\alpha 2\beta 1$ -integrin with collapsed actin. Endorepellin fragment $\Delta 7$ (amino acid residues 4182 to 4391 of perlecan), also called LG3, is described in the specification as filed (specification, Figs. 1f and 1g).

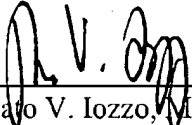
12. Endorepellin Fragment $\Delta 7$ (LG3) Inhibits Branching Morphogenesis and Tube Formation of Human Umbilical Vein Endothelial Cells- Human embryonic cells (293-EBNA cells) were prepared which secrete endorepellin fragment $\Delta 7$ (LG3; SEQ ID NO:10) or endorepellin (SEQ ID NO:3). Tissue culture dishes were prepared with Matrigel™ (BD Biosciences, Bedford, MA) substrates containing endorepellin fragment $\Delta 7$ -secreting or endorepellin-secreting 293-EBNA cells. About 4×10^4 wild-type, endorepellin fragment $\Delta 7$ -secreting, or endorepellin-secreting 293-EBNA cells were cultured in the Matrigel plugs for 24 hours. An equal number of human umbilical vein endothelial cells (HUVECs) were seeded on top of the Matrigel plug and then cultured for 18 hours. Cultures were examined microscopically for tube formation. There was a total inhibition of endothelial branching morphogenesis of HUVECs cultured on the Matrigel substrate containing endorepellin fragment $\Delta 7$ (LG3)-secreting or endorepellin-secreting 293-EBNA cells (see photomicrographic images panels 1B and 1C of Fig. 1, attached), compared to

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HUVECs cultured on Matrigel containing wild type 293-EBNA cells (panel 1A of Fig. 1, attached). It was also found that nanomolar concentrations of endorepellin and endorepellin fragment $\Delta 7$, when briefly applied to collagen-adherent HUVECs (panels 1D and 1E of Fig. 1, attached) or human microvascular dermal endothelial cells (data not shown), caused a significant disruption of actin stress fibers and focal adhesions as visualized by fluorescent imaging. Endorepellin and endorepellin fragment $\Delta 7$ were also found to induce clustering and co-localization of the $\alpha 2\beta 1$ -integrin with collapsed actin.

13. These studies demonstrate that endorepellin and endorepellin fragment $\Delta 7$ are inhibitors of angiogenesis.

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statement and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that willful false statements may jeopardize the validity of the application or any patent issuing thereon.



Renato V. Iozzo, M.D.

2/3/04

Date



DECLARATION- FIGURE 1

